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Assessing the potential risks of transgenic plants for non-target invertebrates in Europe: a review of classification approaches of the receiving environment

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Abstract

According to the current legal background for the regulation of genetically modified plants (GMPs) in Europe, an environmental risk assessment (ERA) has to be performed considering i) the crop plant, ii) the novel trait relating to its intended effect and phenotypic characteristics of the GM crop plant and iii) the receiving environment related to the intended use of the GMP. However, the current GMP-ERA does not differentiate between different intended receiving environments. Therefore, the question is to be raised: How can the 'receiving environment' be classified on the European scale, both in an ecologically relevant and feasible way? As a first step this proposal focuses on invertebrates in the terrestrial environmental compartment. In order to check if already existing regionalization concepts are suitable for the above raised question the following selection criteria were employed:

– Distribution of non-target organisms (NTOs): A suitable regionalization concept should appropriately reflect the specific characteristics of the animal and plant communities of the different receiving environments of a GMP. Therefore, such a classification should be done by an ecoregion approach, meaning that different ecoregions support different organism communities that may play a different role in supporting relevant ecosystem services. However, information on the distribution of invertebrates in Europe is not available in sufficient detail for this purpose. Hence, it is proposed to use the

information about site conditions like climatic, vegetation and soil parameters, which determine the composition of invertebrate communities, for the selection of an appropriate classification concept.
– Size and number of geographical units: This is a trade-off between the total number of 'receiving environments' in Europe manageable in a regulatory context and the ecological uniformity of a single geographical unit. An intermediate size and number of geographical units should be the aim of the classification.

With the 'Indicative map of European biogeographical regions' (IMEBR) there is an existing regionalization concept that meets many of the requirements identified above: the classification is based on parameters that also determine the distribution of invertebrate communities (i.e., the potential natural vegetation) and nine biogeographical regions represented within the 27 member states of the European Union (EU-27) are a manageable number for regulatory purposes. However, epigeic (living above ground) and endogeic (living below ground) faunal communities are determined by different biotic and abiotic parameters. For example, climate data is much more relevant for epigeic species than for endogeic organisms. The most important soil properties related to the distribution of endogeic organisms and plants are pH, texture, organic matter content and/or content of organic carbon, C/N ratio, and water-holding capacity. Hence, for endogeic non-target organisms there is currently no suitable regionalization concept available. For the time being, it is recommended to identify important species for testing purposes in each ecoregion with GMP cultivation by means of expert knowledge using the IMEBR for both epigeic and endogeic communities.

The regionalization concept is intended to be used in the context of the ERA of GMPs for the assessment of risk for NTOs. Hence, it should be tailored for the area in the EU where GMPs are likely to be grown. The overlap between the biogeographical regions and the intended area of cultivation for a novel GMP form the different cases, each of which should undergo a specific ERA process.

For example, there would be eight or nine separate potato cases for the EU-27 area, i.e. the Alpine, Atlantic, Boreal, Continental, Macaronesian, Mediterranean, Pannonian, Steppic and possibly the Black Sea biogeographical regions. For grain maize there would be five to nine separate cases, i.e. the Atlantic, Continental, Mediterranean, Pannonian, Steppic and possibly the Alpine, Black Sea, Boreal and Macaronesian biogeographical regions.

Keywords
Ecoregion, Environmental Risk Assessment, European Union, Genetically Modified Plants, Non-target Organisms

Introduction

Legal and conceptual background

The legal background of the work presented here includes European Union (EU) Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (EC 2001) and the Cartagena-Protocol on Biosafety (CBD 2000). According to both documents, the environmental risk assessment (ERA) of genetically modified plants (GMPs) must be performed on a case-by-case basis. In Annex II of Directive 2001/18/EC, a case is defined as a combination of the crop plant (its biology, ecology and agronomy), the novel trait relating to its intended effect and phenotypic characteristics of the GM crop plant (i.e., the whole GMP), and the receiving environment related to the intended use of the GMP. However, the current practice of ERA does not differentiate between different receiving environments of a GMP (Romeis et al. 2008). Therefore, the question is to be raised: How can the receiving environment of GMPs be classified on the European scale, both in an ecologically relevant and feasible way? Ecologically relevant in this context means, that there should be a close relationship between the protection goal, i.e. the conservation and sustainable use of biological diversity in the likely potential receiving environment (CBD 2000) and the methodology used to assess the potential adverse effects of GMP on this protection goal in a specific case. Feasible means that it must be possible to complete the ERA on such a time and resource scale that the need to perform the process itself will not be the knock-out criterion for the development of novel GMPs.

The overall problem to be addressed is the improvement of the ERA of GMPs for non-target organisms (NTOs), in particular in the context of the case-specific selection of test species (Hilbeck et al. 2011a; 2011b). Hence, this contribution focuses on invertebrate groups which are currently used in the ERA. Other groups, e.g. vertebrates like amphibians, reptiles and birds, may be addressed later. In addition, only terrestrial environments will be discussed.

Any suitable concept for the classification of different receiving environments of GMPs should appropriately reflect the specific characteristics of their invertebrate communities. Therefore, such a classification should be done by an ecoregion approach, meaning that different ecoregions support different organism communities that may play a different role in supporting relevant ecosystem services. Thus, it would be desirable to base the classification mainly on the composition of communities. In this case, detailed information on the distribution of a wide range of organisms within Europe is required to derive areas of similar community composition (ecoregions) leading to a biogeographical classification system.

Biotic and abiotic factors affecting the distribution of NTOs

The occurrence and distribution of plant species and plant communities is well known for many parts of Europe (e.g., Ellenberg et al. 1992; Beck et al. 2005). In addition, the potential natural vegetation (PNV), i.e., the vegetational climax stage which would occur if there is no anthropogenic influence, has already been widely mapped (Hornsmann et al. 2008). While for some above-ground invertebrates, such as ground beetles, data exists (Trautner and Geigenmüller 1987), for most invertebrates and in particular soil organisms, this information does not exist. Thus, it is currently not possible to use real distribution data of these invertebrates for the definition of a biogeographical classification concept. For this reason it has been proposed to use the spatial characteristics of factors, which determine the occurrence of plants and animals, instead (Ghilarov 1965; Breure et al. 2005). The distribution of single species and the composition of organism communities at any given site are determined by the local biotic and abiotic conditions. For example, the correlations between soil parameters such as pH value or texture (percentage of sand, silt and clay particles) and the occurrence of specific organism group compositions, which have been hypothesized for a long time (Volz 1962; Ghilarov 1965), was successfully used in recent classification concepts developed in the Netherlands (Biological Indicator for Soil Quality; BISQ) and Germany (Biological Soil Classification and Assessment Concept; BBSK) (Römbke and Breure 2005). In the following, the most important factors are presented.

Climatic parameters, in particular precipitation, elevation, soil and air temperature as well as their interactions are some of the main factors relevant for the distribution of many organisms (e.g., Ellenberg et al. 1986). Their impact depends strongly on their time course as well as on 'extreme' values and much less on mean values. Hence, it is difficult to use the available information directly for the prediction of the distribution of species or the composition of communities. In general, climate data is much more relevant for epigeic (living above ground) species and primarily plants than for endogeic (living below ground) organisms. However, due to its overall impact in setting the stage for the formation of specific organism communities climate needs to be taken into account when trying to define different biogeographical regions in general.

Vegetation is an important factor for the distribution of many organisms. However, especially at agricultural sites the actual vegetation permanently changes and there are many other anthropogenic influences at any given site. On the other hand, the PNV is the 'climax' vegetation that will develop at a site through natural succession without anthropogenic disturbance, natural disaster or gradual climatic change (Härdtle 1989). The PNV is an expression of environmental factors such as topography, parent rock material and climate across an area. Twenty years ago, a 'Map of Natural Vegetation of the member countries of the European Community and of the Council of Europe' was produced (Noirfalise 1987). More recently, the German Federal Agency for Nature Conservation (BfN) produced a 'Map of the Natural Vegetation of Europe'. A current version of this map is shown in Fig. 1. For epigeic organisms, the PNV may be regarded to be the primary factor determining their distribution (e.g., for carabid beetles: Thiele and Kolbe 1962; Thiele 1977; Vogel and Krost 1990; Scheurig et al. 1996).

The most important soil properties related to the distribution of endogeic organisms like Collembola or earthworms are pH value, texture, organic matter (OM) content and/or content of organic carbon and C/N ratio (Dunger 1998; Römbke et al. 2002; Breure et al. 2005), but not directly the occurrence of certain plant species. In fact, many species can be found in soils covered by very different types of vegetation (Dunger and Dunger 1983). For example, the most widespread European earthworm species (e.g., *Aporrectodea caliginosa, Aporrectodea rosea, Lumbricus terrestris, Lumbricus rubellus, Lumbricus castaneus*) are found in different types of grasslands or forests, given that soil conditions and food resources are favourable (e.g., Margerie et al. 2001; Römbke et al. 2005). Soil moisture is also highly relevant but due to its variability and delayed effect it is almost impossible to measure and evaluate its relevance for the distribution of soil organisms. Bulk density of the soil may also be an important factor

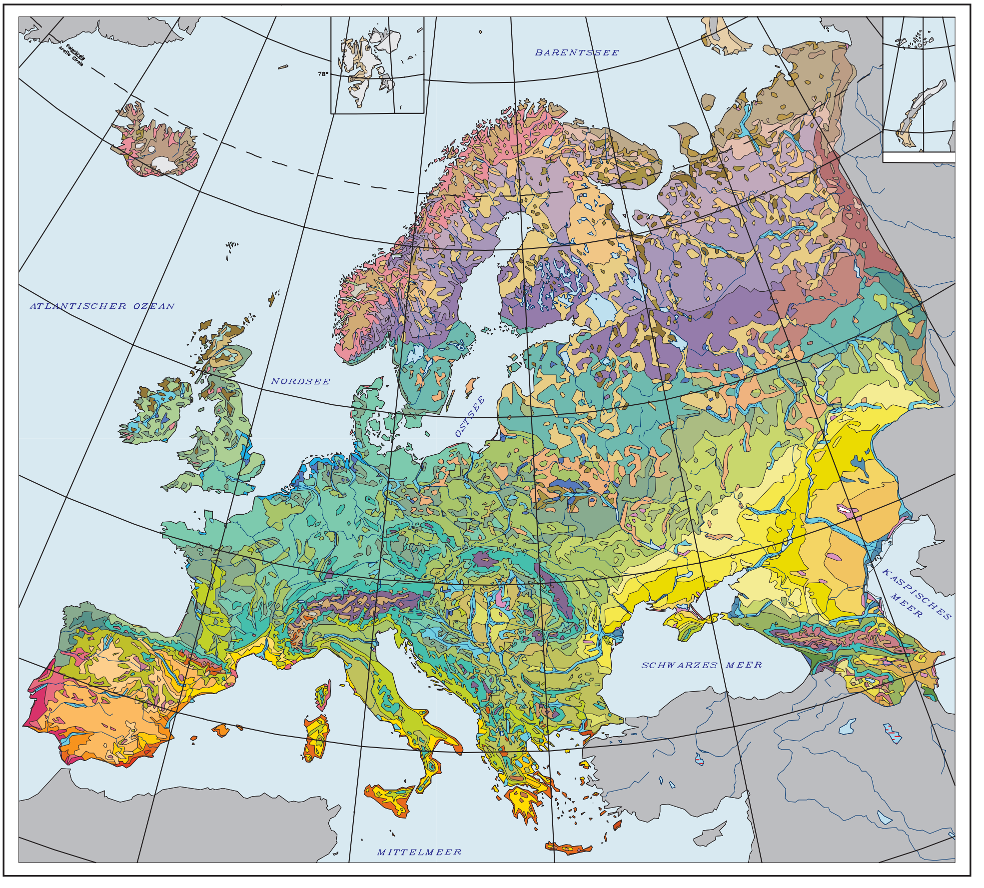

Figure 1. Natural vegetation of Europe. The different colours represent the various vegetational cartographic units. The number of classes is too large to be listed here. For details see Bohn et al. (2002/2003).

but very little information on its impact on soil organism distribution on a larger scale is available. Most recent studies are concerned with the impact of local soil compaction due to human activities such as different farming practices. Soil type (e.g. Albeluvisol or Cambisol, the most common soil types in Europe; EC 2005) is also not suitable for biogeographical classification purposes, since a similar soil type, i.e. soils which show comparable horizons and often share a common history, may not necessarily have the same properties, nor host the same communities of soil organisms (Schröder et al. 2001). Maps for the spatial characteristics of factors being relevant for the distribution of soil organisms like the sand content or the organic matter content are already available (EC 2005). Currently, a map showing pH-values (probably the most important factor for many soil organisms) of European soils is under preparation by the European Soil Bureau (Ispra, Italy). However, it must be stated that ultimately no single factor but the combination of various factors (i.e., pH, texture, OM content, etc.) determines the composition of specific communities.

Additionally, factors that may explain occurrence patterns for terrestrial organisms that would not be understandable when only looking at the actual soil and climate properties need to be considered as well. For example, historical events like the glaciation (and thus soil destruction) of Northern Europe in the Quaternary (2.58 Ma to present) help to explain the current distribution pattern of earthworms (Stop-Bøwitz 1969).

Based on this knowledge, it is proposed to use the information about site conditions (including climatic, botanical and soil parameters), which determine the composition of communities, for the biogeographical classification of the distribution of terrestrial organisms. Due to their species richness, ecological relevance and especially their role as NTOs, invertebrates will be the main focus within the ERA of GMPs. Therefore, the basic hypothesis can be formulated as follows (see also EFSA 2010a):

Europe can be divided into a number of ecoregions defined by a combination of abiotic and biotic factors relevant to the distribution of terrestrial organisms, e.g. climate, PNV, soil properties and other factors like site history.

Each region supports a different community that may play a different role in supporting ecological services like organic matter decomposition (even the extent of this support may differ: i.e. without vertical burrowing earthworm species the influence of soil organisms on soil structure is smaller as in those regions where these species occur).

Aim of the review

The aim of this review is not to develop a new biogeographical classification concept but to identify and evaluate the suitability of already existing regionalization concepts for Europe for the classification of the receiving environment within the ERA of GMP using a number of selection criteria. Realizing the very different ecological requirements between and within large organism groups like plants and epigeic and endogeic invertebrates it is likely that it will not be possible to identify one biogeographical classification concept for Europe which is equally relevant for all of them. However, the aim has to be to find a compromise which is as representative as possible for the organism groups discussed here, i.e. terrestrial invertebrates.

Methods

Selection of the regionalization concept

A literature/web based research on European biogeographical classification was performed. The identified concepts were evaluated according to their suitability for the classification of the receiving environment within ERA of GMPs using the following selection criteria.

Number and uniformity of biogeographical units: This criterion relates to the feasibility of using the classification for the ERA process. A balance between a manageable number of regions in a regulatory context and the ecological uniformity of a single biogeographical unit must be achieved. Thus, the selected units must be smaller than just 'Northern' or 'Southern' Europe but larger than a single agricultural field. Hence, an intermediate size of the biogeographical unit should be the aim of the classification. It is important to agree on a total number of units for Europe, which makes the ERA-process manageable in terms of time and cost but also allows for scientifically acceptable risk estimation. For example, in efficacy trials for plant protection products 10 (range: 6 to 15) different trials across the range of climatic and environmental conditions likely to be encountered are recommended (EPPO 2004a; 2004b). Accordingly, the same order of magnitude seems to be reasonable for the number of different biogeographical regions in the context of the ERA for GMPs as well. For this review a threshold value of 20 was used meaning that any regionalization concept with more than 20 biogeographical units was considered as unsuitable for the ERA of GMPs. A minimum number of regions was not determined but the biogeographical units should allow for a sufficient differentiation of different terrestrial invertebrate communities.

Geographical coverage of the European Union: The EU Directive 2001/18/EC applies to all member states and hence currently the area of the EU-27. Accordingly, any regionalization concept suitable for the ERA of GMPs in the EU should cover this entire area.

Coverage of ecologically relevant factors: As outlined above, the abiotic and biotic factors of a given site determine its invertebrate community composition. Hence the identified regionalization concepts were checked as to whether or not the following factors were considered in the derivation of their biogeographical units:

- climate (e.g., precipitation and temperature);
- actual vegetation or PNV;
- soil factors (e.g., pH value, OM content and texture).

The inclusion of climatic or vegetational factors indicates a high relevance for epigeic invertebrates while soil factors are more relevant for endogeic invertebrates.

Ecological focus of the regionalization concept: Regionalization concepts proposed so far were most likely developed for a very specific purpose such as the fulfillment of regulatory requirements. This means that even when factors such as climate or soil factors are included in the derivation of the concept, its goal can be different which has consequences when trying to apply the concept in a different context, i.e. the ERA of GMPs. It is assumed that it would be an advantage for this transfer step if the regionalization concept of choice was already developed with a focus on an ecological topic.

Current political relevance: Any newly proposed regionalization concept for the ERA of GMPs will need a broad acceptance by legislators in order to get implemented. For this reason it would be helpful if the regionalization concept was already in use for other regulatory purposes. This way practicability would be demonstrated more easily and existing experience could facilitate its introduction into the regulatory context of GMPs.

Application of the regionalization concept for specific GMPs

Since the biogeographical regionalization concept is to be used in the context of the ERA of GMPs, it should be tailored for the very area in the EU where GMPs are likely to be grown. Hence, the distribution of agricultural crops in Europe should be considered. The biogeographical regions of the regionalization concept of choice correspond to the different potential receiving environments for any given GMP in the EU-27 area. The overlap between these generic receiving environments and the prospective areas of cultivation for a novel GMP form the different cases, each of which should undergo a specific ERA process. Data on cultivation areas of crops whose transgenic lines are currently approved in the EU (potato and grain maize) are available on the NUTS (Nomenclature of Territorial Units for Statistics) 2 (UK: NUTS 1) level (Figs. 2 + 3). This data illustrates that these crops are widely spread throughout Europe. Potato and grain maize were also chosen as example crops for GMP cases considered for an expert workshop on species selection procedure due to pending applications for cultivation of GM potato and GM maize lines in the EU (Hilbeck et al. 2011a; 2011b).

Results and Discussion

Existing European classification concepts

Zones of comparable climate in the EPPO region: The European and Mediterranean Plant Protection Organization (EPPO) aims to harmonize the efficacy evaluation of plant protection products in its member countries. Its standards describe how field trials should be conducted to generate useful data for registration purposes. The scope of EPPO guideline PP 1/241 (1) (EPPO 2005) in particular is to provide guidance to regulatory authorities and applicants on the comparability of climatic conditions within the EPPO region. It describes four different climatic zones, in which climatic conditions may be considered comparable (Fig. 4).

This classification is much too rough for the purpose of the ERA of GMPs. The borders of EPPO regions are, with the exception of the Mediterranean zone, determined by political structures, i.e. the borders of national states. This shows that climatic differences were not the most important criterion of this classification. The EPPO guideline itself recognizes that there are other important factors in establishing the relevance of data generated for efficacy evaluation in one region for another region, e.g. soil properties such as organic matter content, pH and moisture content. These factors as well as vegetation were not considered in the derivation of the four regions.

Thus, although the concept has an ecological focus and is used in a regulatory context it is not considered suitable for the classification of the receiving environment within ERA of GMPs (Table 1).

FOCUS-Concept: FOCUS (Forum for the co-ordination of pesticide fate models and their use) is an initiative of the European Commission to harmonize the calculation of predicted environmental concentrations (PEC) of active substances of plant protection products (PPP) in the framework of the EU Directive 91/414/EEC (EC 1991). FOCUS is based on co-operation between scientists of regulatory agencies, academia and industry. In 1997, the Soil Modeling Work group of FOCUS developed eight scenario regions for Europe based on net precipitation and average temperature (Van der Linden et al. 1997; Fig. 5). This classification aimed to improve the modeling and, thus, the assessment of the fate of pesticides by referring to the range of soil and climate properties in Western Europe. The scenario regions were characterized regarding their areal percentage of arable land, their dominant soil types, relative abundance of texture classes and organic matter within dominant arable land areas and dominant crops. This approach, focusing on the groundwater compartment, is an important tool in the ERA of pesticides (Boesten et al. 1997). However, it has several shortcomings:

– it does not cover the current area of the European Union;
– it does not include vegetational data;
– it does not have an ecological focus.

Despite these shortcomings this classification concept might become useful for the classification of the receiving environment within the ERA of GMPs in the future (Table 1). It is currently being revised through working groups of the European Food Safety Authority (EFSA) and the European Soil Bureau (ESB) aiming to include biological and soil parameters. For this purpose a database will be developed covering the occurrence of major soil organism groups in Europe (EFSA 2010a). However, this revision focuses only on three countries of the EU and, thus, cannot be considered here in more detail.

Indicative map of European biogeographical regions (IMEBR): The first version of the European Council Directive 92/43/EEC (EC 1992/1995) on the conservation of natural habitats and of wild fauna and flora (Habitats Directive) identified five biogeographical regions as the framework for setting up the Natura 2000 ecological network (Special Areas of Conservation; SACs). However, the Directive lacked a clear definition of these regions. Starting with the aforementioned maps of PNV for Europe (e.g., Fig. 1), several versions of the biogeographical regions map were produced. The main steps leading from the maps of PNV to the biogeographical regions are described in ETC/BD (2006). The current map of the Pan-European biogeographical regions (including Asia Minor) now defines 11 regions, of which 9 are represented in the member states of the EU-27 (Fig. 6).

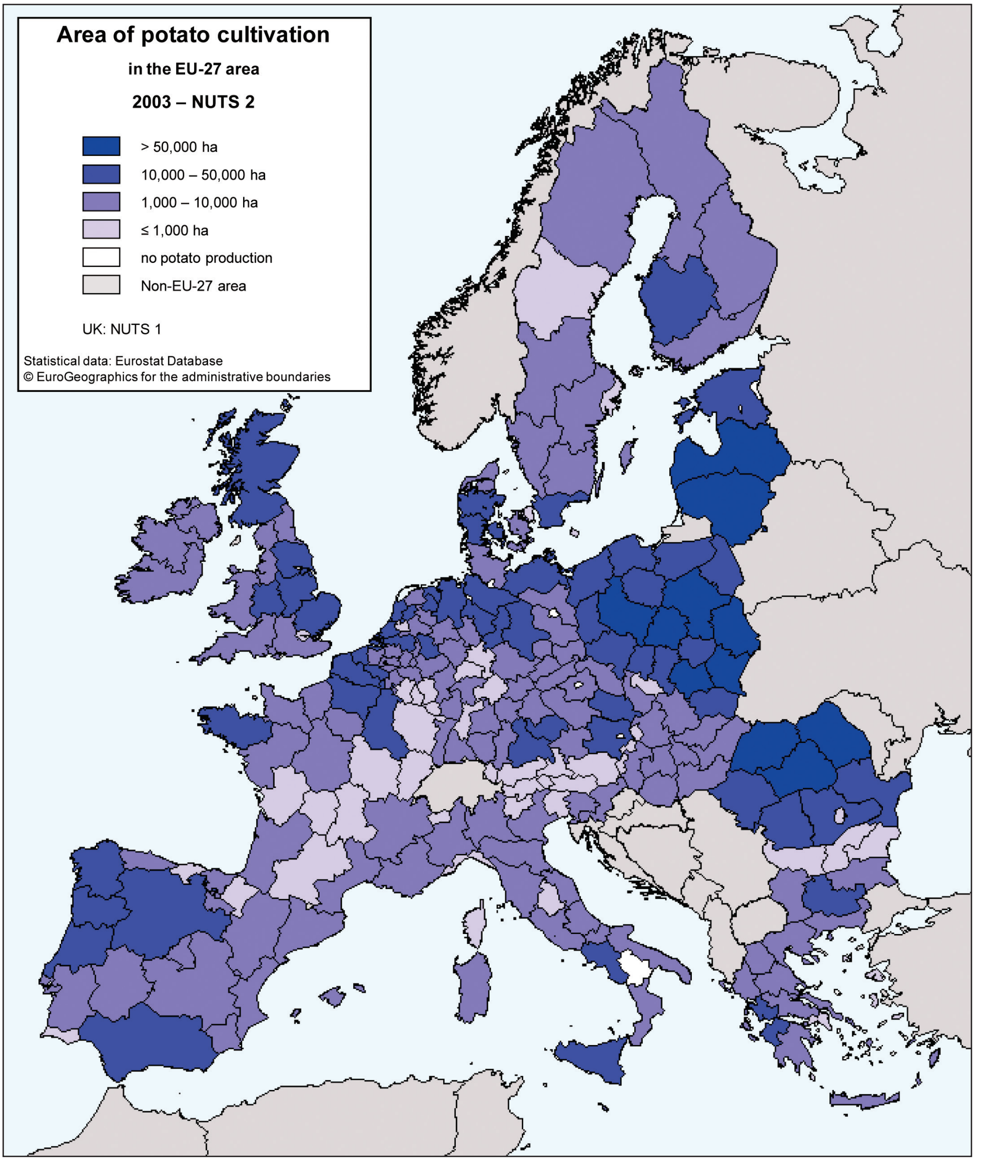

Figure 2. Area of potato cultivation in the EU-27 area.

The IMEBR is based on data on the PNV and thus indirectly also climate. Hence, it relies on what is generally considered the most important factors for the distribution of epigeic organisms. It is an internationally recognized regionalization concept aimed at the harmonization of nature conservation in Europe and thus has an ecological scope. It is also considered in the draft scientific opinion 'guidance on the environmental risk assessment of genetically modified plants' of the EFSA Panel on Genetically Modified Organisms (EFSA 2010b). Additionally, the total number of nine regions in the EU-27 area can be considered a practical differentiation of the receiving environ-

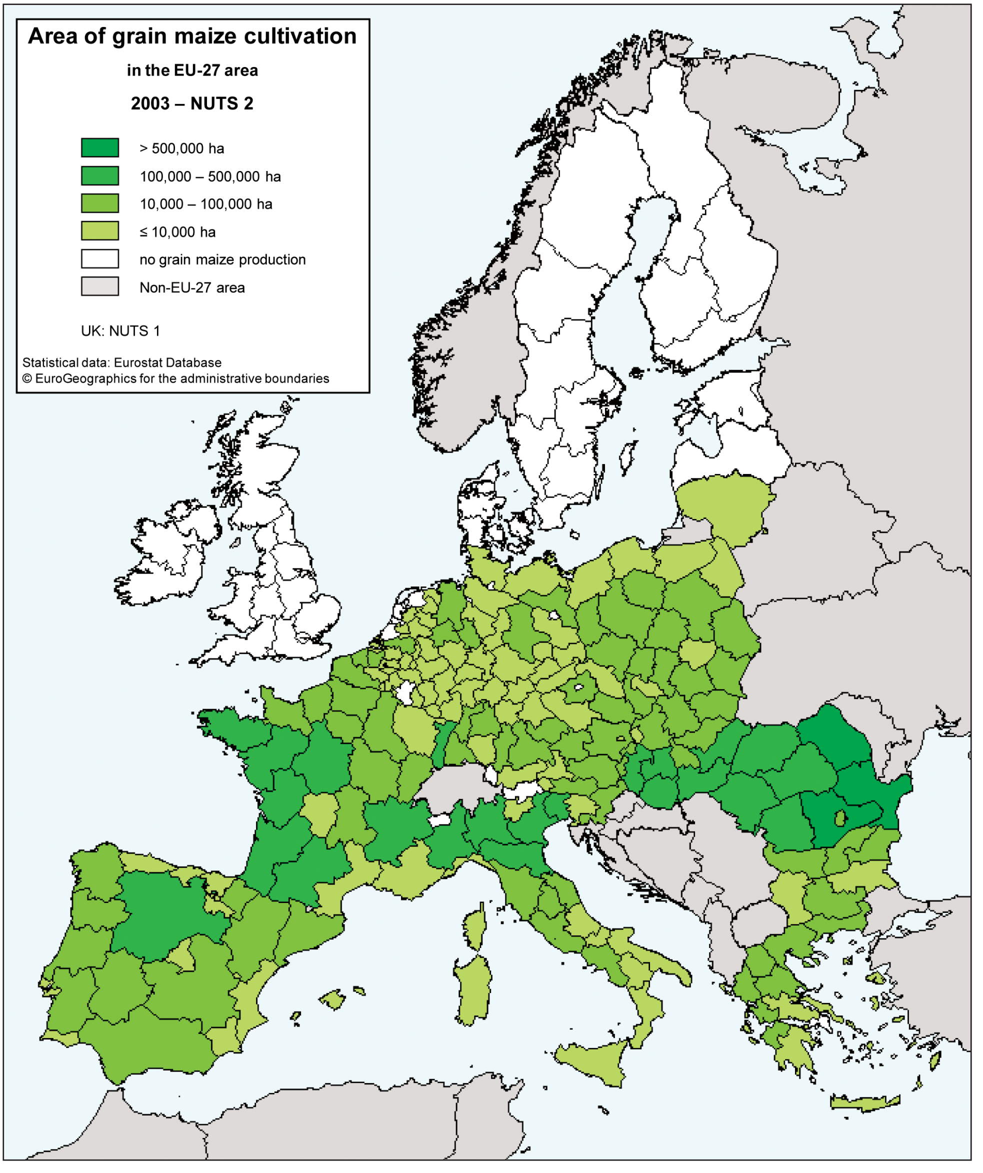

Figure 3. Area of grain maize cultivation in the EU-27 area.

ment within the ERA of GMPs in the EU Thus, it has a high potential for the use in future ERA concepts for GMPs (Table 1). However, it is important to stress, that this does not extend to the endogeic invertebrate community, since soil properties have not been considered in this classification concept so far.

Ecological land classification of Europe (ELCE): Hornsmann et al. (2008) developed a European ecological land classification that is aimed at the optimization of European environmental monitoring networks. The land classification was calculated

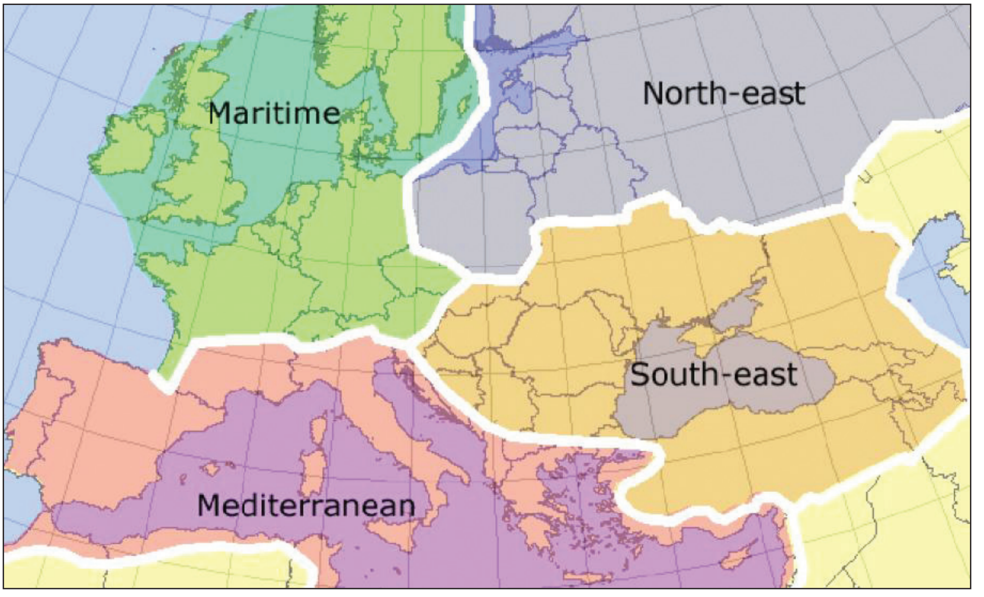

Figure 4. Zones of comparable climate in the EPPO region, for the purposes of efficacy evaluation trials on plant protection products (EPPO 2005). Efficacy evaluation of plant protection products. Guidance on comparable climates. EPPO standard PP 1/241 (1). EPPO Bulletin 35: 569-571 by EPPO (European and Mediterranean Plant Protection Organisation). Copyright 2005 by John Wiley and Sons. Reproduced with permission of John Wiley and Sons via Copyright Clearance Center.

from surface data on the potential natural vegetation, soil texture, elevation and climate (Weustermann et al. 2009). In its least detailed differentiation level, Europe is divided into 40 (most detailed: 200) ecoregions (Fig. 7). This classification concept takes into account (almost all) factors which determine the distribution and composition of epigeic and endogeic faunal communities. It was developed for monitoring purposes and thus has an ecological focus. However, even the least detailed differentiation yields a number of classes for Europe that cannot be considered practical for the ERA of GMPs (Table 1).

Digital map of European ecological regions (DMEER): Comparable to Hornsmann et al. (2008), 68 ecological regions based on knowledge of climatic, topographical and geobotanical (i.e., the natural vegetation of Europe) data have been defined by the European Topic Centre on Nature Protection and Biodiversity (ETC/BD) with the judgement of a large team of experts from several European nature related institutions and the World Wide Fund For Nature (WWF) (EEA 2003; Fig. 8). The map is aimed at showing the extent of areas with relatively homogeneous ecological conditions, within which comparisons and assessments of different expressions of biodiversity are meaningful to improve European efforts to assess, monitor, plan and share ecological data. This classification concept has an even finer resolution than the concept by Hornsmann et al. (2008) and hence cannot be considered practical for the ERA of GMPs either (Table 1).

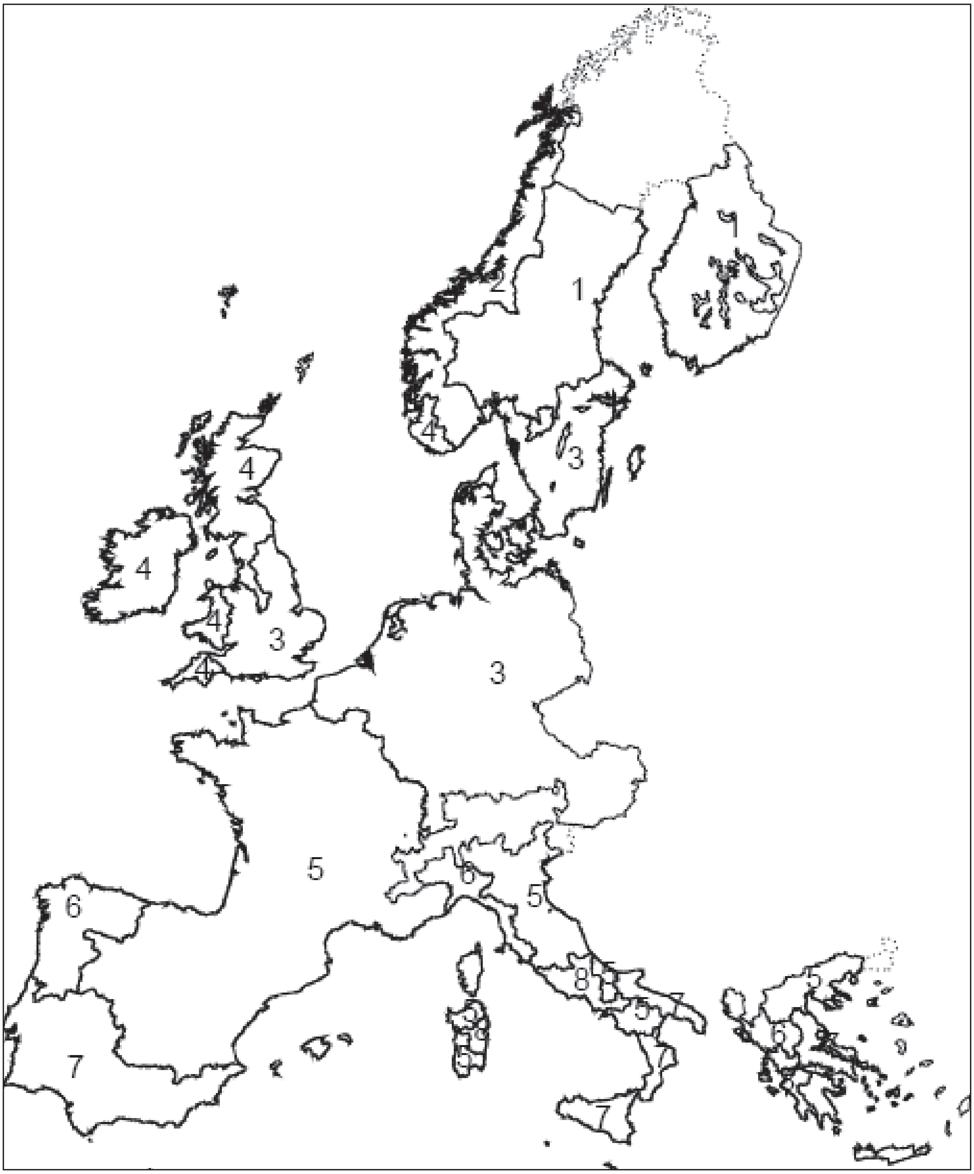

Figure 5. FOCUS-Scenario regions in Europe (Van der Linden et al. 1997): 1 = Central Sweden, Finland; 2 = Norway; 3 = South Sweden, South-East England, Denmark, Belgium, Netherlands, Luxembourg, Germany, Elzas (France); 4 = Ireland, Scotland, North England, West England, Wales; 5 = France, North-East Spain, North-Central Italy, Sardinia, West Corsica, North East Greece; 6 = North-West Italy, North-West Greece, North Portugal, North-West Spain; 7 = South-Spain, South Portugal, South-Italy, Sicily, South-East Greece; 8 = East Corsica, Central Italy, West Greece.

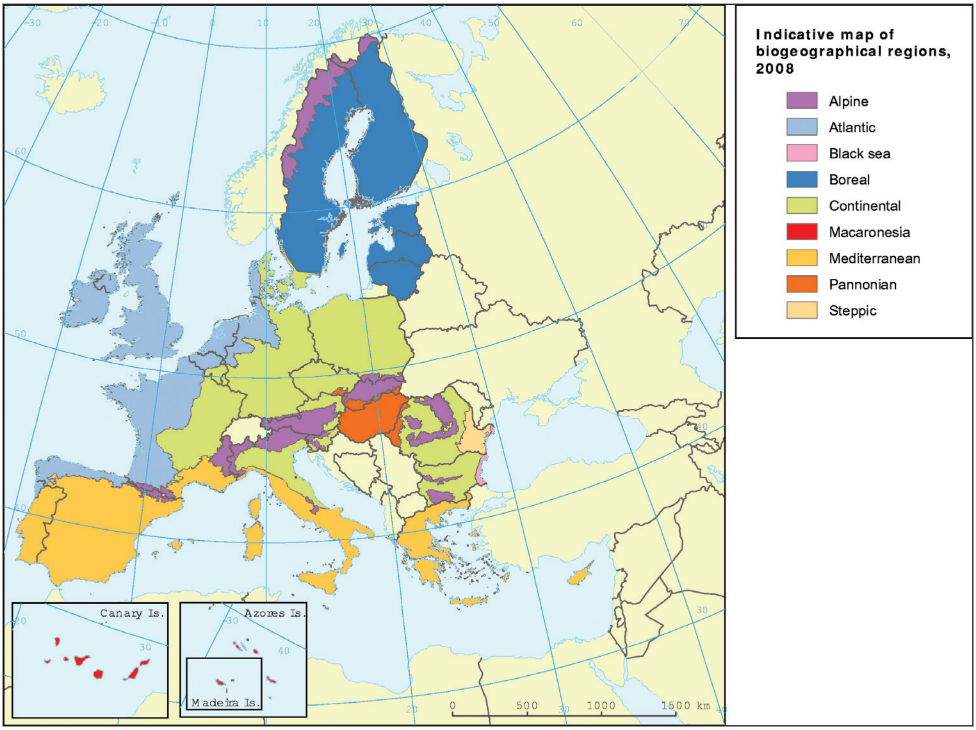

Figure 6. Indicative map of the EU-27 biogeographical regions (EEA 2009).

Choice of the regionalization concept

As outlined above, epigeic and endogeic faunal communities are determined by different biotic and abiotic parameters. The ELCE concept (Hornsmann et al. 2008) that takes all of these parameters into account leads to a regionalization too finely differentiated with too many different biogeographical regions to be practical for the GMP regulation process. Of all the identified regionalization concepts, the IMEBR (ETC/BD 2006) appear to be the most suitable for the ERA of GMPs (Table 1). However, regarding its determining factors, it is currently mostly applicable for epigeic organisms. For endogeic NTOs there is currently no clearly suitable regionalization concept available. For this reason it is recommended, that for the time being the receiving environments of a given GMP in Europe will be classified using the IMEBR. From a regulatory perspective, the nine biogeographical regions represented within the member states of the EU-27 are currently relevant (Fig. 6). In the near future this concept should be revised, e.g. through a scientific expert workshop, in order to evaluate to what extent the current concept needs to be amended to adequately consider the differences in the endogeic organism communities within the already defined biogeographical regions. In order to include biological and soil parameters, a database is being developed covering the occurrence of major soil organism groups in Europe. The revision of the

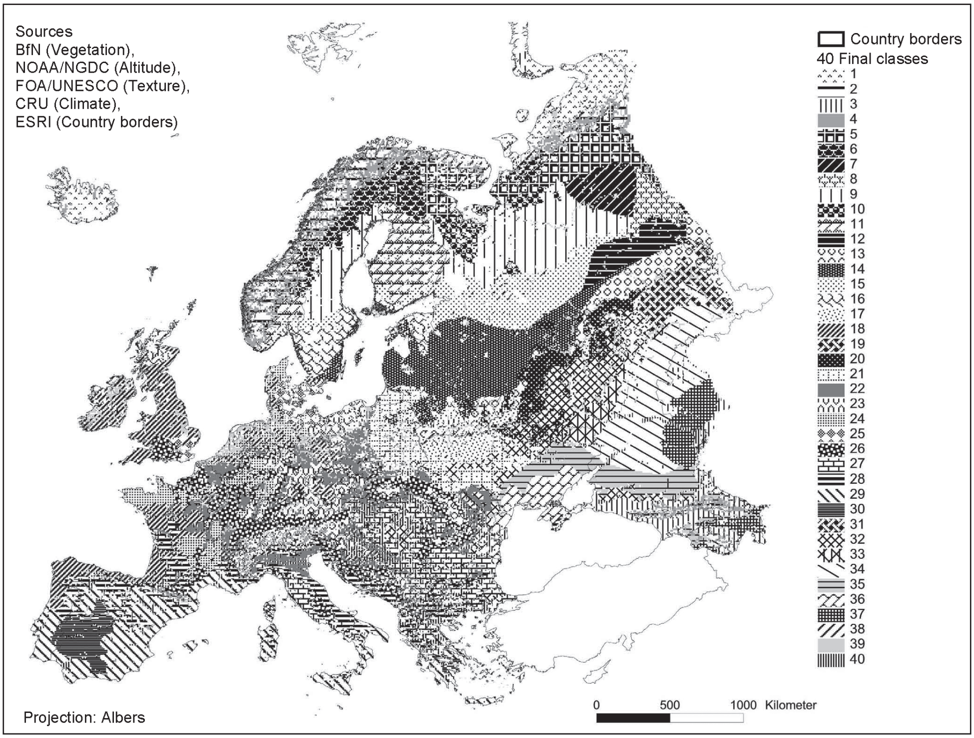

Figure 7. Ecological land classification of Europe (ELCE). 40 ecoregions based on potential natural vegetation, soil texture, elevation and climate (Hornsmann et al. 2008). With kind permission from Springer Science+Business Media: Umweltwissenschaften und Schadstoff-Forschung – Zeitschrift für Umweltchemie und Ökotoxikologie, Berechnung einer landschaftsökologischen Raumgliederung Europas, Vol. 20, 2008, pp. 25–35, I. Hornsmann, G. Schmidt, W. Schröder, figure 1.

IMEBR might lead to a further sub-division of some of these biogeographical regions, e.g. by incorporating the effects of glaciation during the last ice age on the geographical distribution of soil organisms, in particular earthworms. However, it should be verified that the total number of regions will not become too high to be practical.

Application of the regionalization concept for specific GMPs

Fig. 9 illustrates the overlap between the area of cultivation of potato and grain maize and the nine biogeographical regions of the EU-27 area. The only NUTS-2 unit without potato cultivation (except for some larger cities) is the region of Basilicata in Southern Italy. Thus, potato is cultivated within all EU-27 biogeographical regions (data for the Macaronesian region not shown). However, the resolution of the NUTS-2 units is not sufficient to clarify if there is in fact potato cultivation in the Black Sea region. Hence, assuming that the transgenic isoline for potato was to be cultivated in all of Europe, there would be eight or nine separate potato cases to be included in the ERA

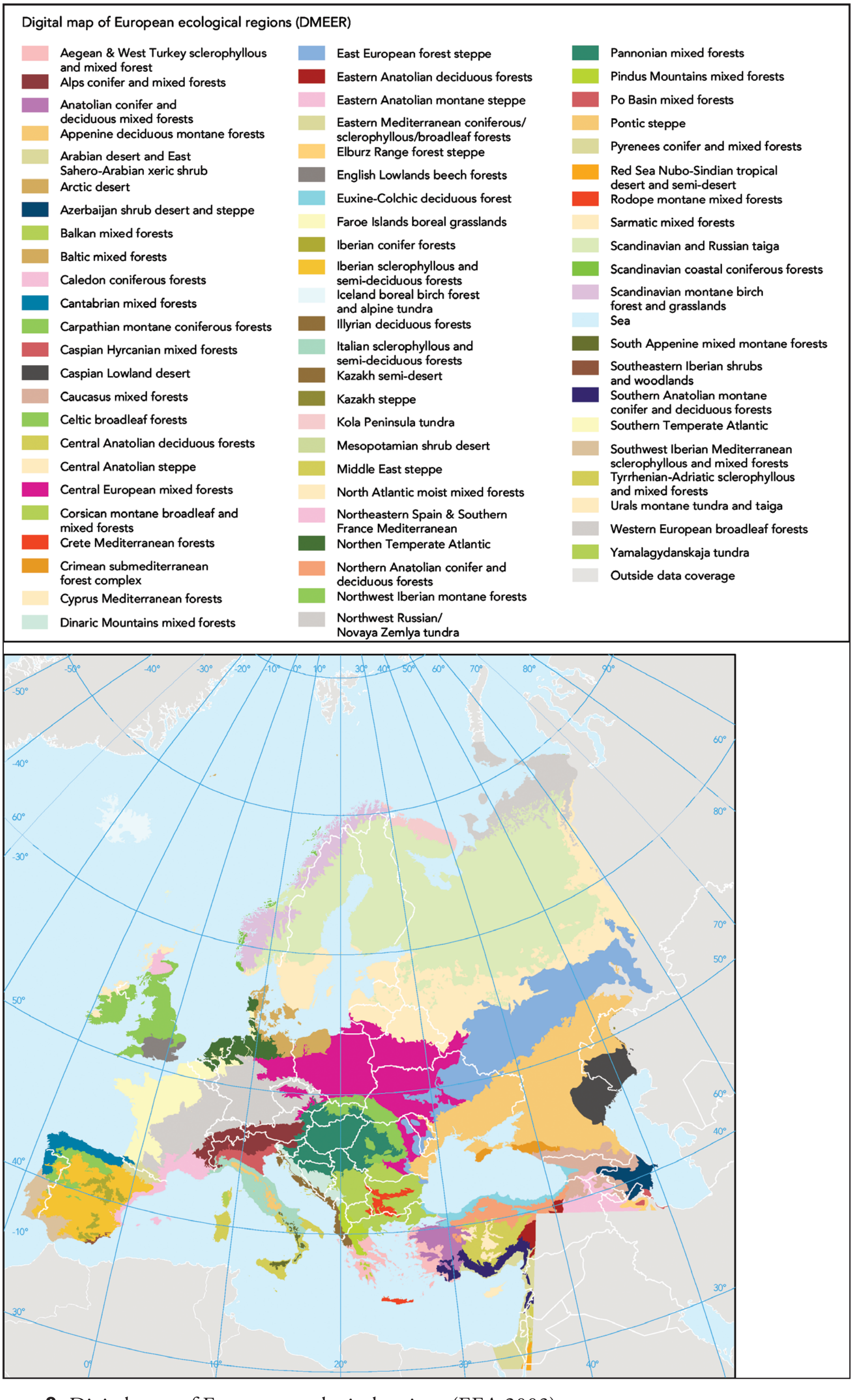

Figure 8. Digital map of European ecological regions (EEA 2003).

Table 1. Evaluation of the suitability of different European regionalization concepts for the classification of the receiving environment within the ERA of GMPs.

	EPPO	FOCUS	IMEBR	ELCE	DMEER
Number/uniformity of regions	-	+	+	-	-
Geographical coverage of EU	+	-	+	+	+
Coverage of ecological relevant factors:					
- Climate	+/-	+	+/- *	+	+
- Vegetation/PNV	-	-	+	+	+
- Soil factors	-	+	-	+	-
Ecological focus	+/-	-	+	+	+
Current political relevance	-	+	+	-	-
Suitable for the ERA of GMP	No	With restrictions	With restrictions	No	No

* Indirectly included through PNV.

for the EU-27 area. Grain maize cultivation is mainly limited to Central, Eastern and Southern Europe. There is no grain maize cultivation in Ireland, Great Britain, Denmark, Sweden, Finland, Estonia, Latvia and some NUTS-2 units of the Netherlands, Belgium, Austria and Italy. Thus, grain maize is also cultivated within all biogeographical regions of the EU-27 area (data for the Macaronesian region not shown). However, cultivation within the Boreal region is limited to a relatively small area in Lithuania and there is also very little grain maize cultivation in the Macaronesian region, i.e. the island of Madeira, the Azores and the Canary Isles. Also, the resolution of the NUTS-2 units is not sufficient to clarify if there is indeed grain maize cultivation in the Alpine and Black Sea regions. Hence, there would be five to nine separate grain maize cases to be included in the ERA for the EU-27 area. The ultimate number of cases for any given GMO will depend on the actual area for which release is requested by the applicant which will in turn depend on a number of factors such as the distribution of pests or economic considerations. In regulatory practice it might also prove useful as an initial step, to take into account the differing proportions of a specific crop in the different regions (cf. Figs. 2+3), leading to a density-dependent prioritization of the overall ERA process. However, due to the biological potential of GMPs to spread and reproduce as well as the possibility of horizontal and vertical gene transfer, there is no acceptable threshold, below which the proportion of a GMP cropped area may be considered negligible. Hence, such a prioritization step must not lead to an overall omission of regions with only a low cropping area of the concerned transgenic crop.

Use of the regionalization concept for the selection of test species

The selection of test species would have to follow this regionalization concept for both epigeic and endogeic NTOs, with a clear understanding of the short-comings for endogeic species. When doing so, either individual species representing these communi-

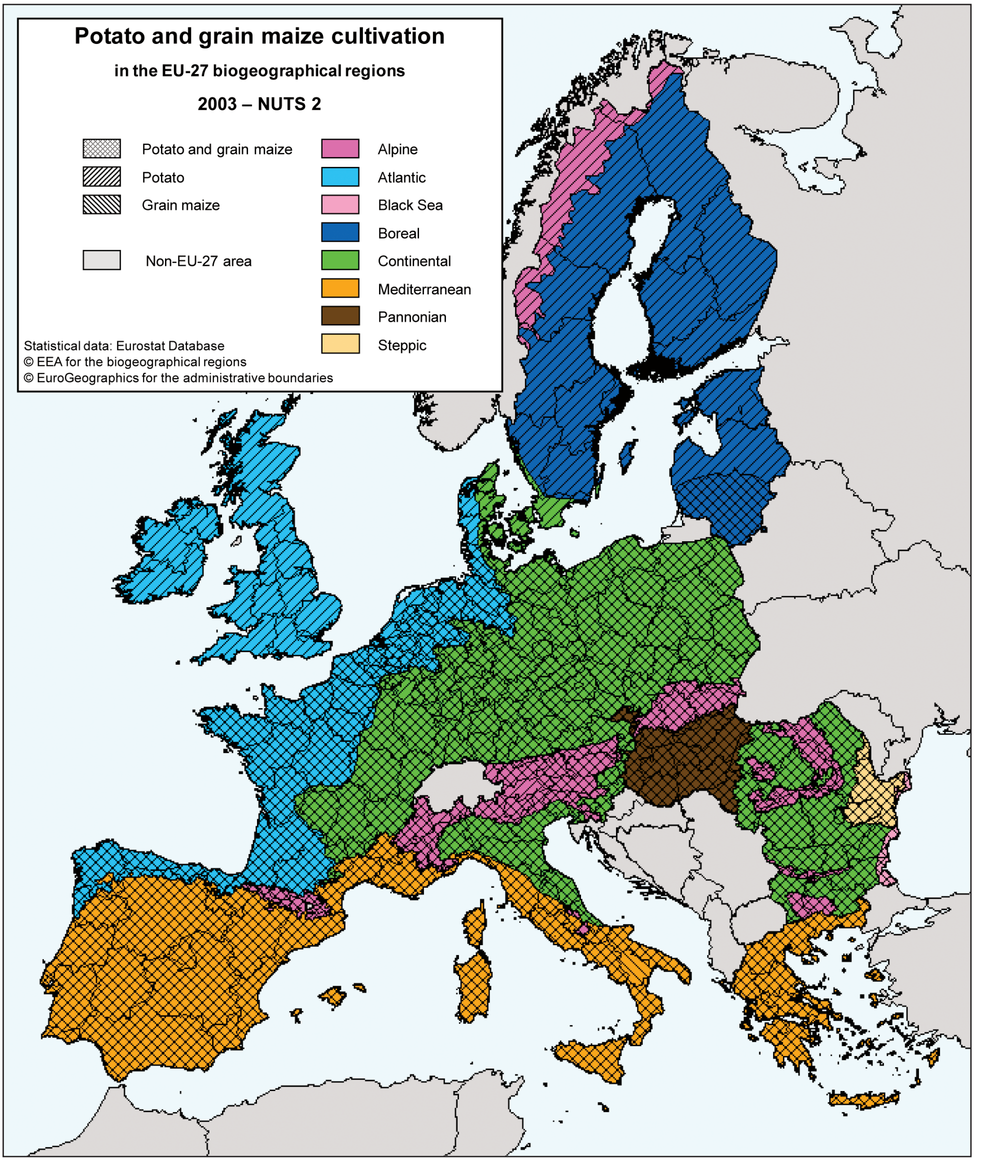

Figure 9. Overlap between the area of cultivation of potato and grain maize and the EU-27 biogeographical regions.

ties or species being of outstanding ecological relevance (e.g., ecosystem engineers; Jones et al. 1994) have to be identified on a case-by-case basis (Hilbeck et al. 2011a, 2011b). As far as possible standard test methods should be chosen in order to improve comparability, practicability and data acceptance. However, it is also clear that in order to use ecologically relevant species occurring in a specific region in which the GMP to be assessed is or will be grown, different test species (e.g. for the soil compartment, the compost worm *Eisenia fetida* would not be sufficient). An overview on the criteria for the identification of test species and methods can be found in Römbke et al. (2009).

Conclusions

With the IMEBR there is an internationally recognized regionalization concept that is potentially suitable for the classification of the receiving environment within the ERA of GMP, in particular regarding epigeic invertebrates. For endogeic invertebrates this concept will have to be modified in the near future. A potentially useful concept for this purpose has been developed by EFSA and ESB working groups in the context of pesticide ERA, but it is not available for the EU-27 yet. For the time being, it is recommended to identify important species for testing purposes in each ecoregion with GMP cultivation by means of expert knowledge using the IMEBR for both epigeic and endogeic communities.

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